



Europäisches Patentamt
European Patent Office
Office européen des brevets



(11)

EP 1 488 816 A1

(12)

EUROPEAN PATENT APPLICATION

published in accordance with Art. 158(3) EPC

(43) Date of publication:
22.12.2004 Bulletin 2004/52

(51) Int Cl.7: **A61L 27/38, C12N 5/00**
// C12M3:00

(21) Application number: **03707196.6**

(86) International application number:
PCT/JP2003/002501

(22) Date of filing: **04.03.2003**

(87) International publication number:
WO 2003/074100 (12.09.2003 Gazette 2003/37)

(84) Designated Contracting States:
AT BE BG CH CY CZ DE DK EE ES FI FR GB GR
HU IE IT LI LU MC NL PT RO SE SI SK TR

(72) Inventor: **SUZUKI, Shigeki**
Setagaya-ku, Tokyo 154-0001 (JP)

(30) Priority: **04.03.2002 JP 2002057129**

(74) Representative: **Grünecker, Kinkeldey,**
Stockmair & Schwanhäusser Anwaltssozietät
Maximilianstrasse 58
80538 München (DE)

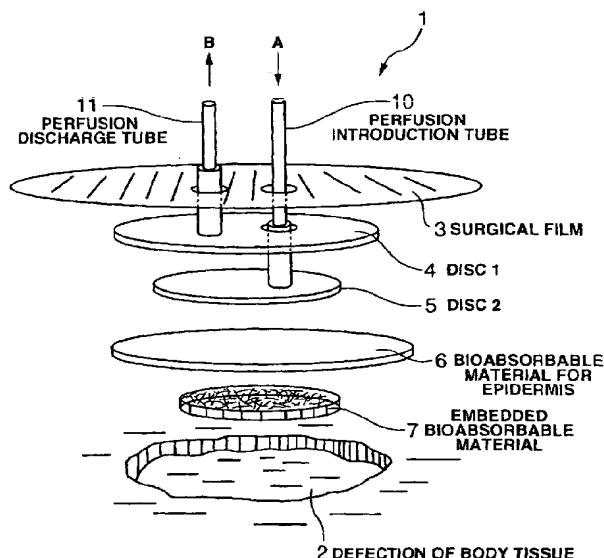
(71) Applicant: **New X-national Technology K.K.**
Tokyo 150-0002 (JP)

(54) CLOSED CELL CULTURE SYSTEM

(57) An object of the present invention is to provide a tissue cell culture system whereby a call tissue can be efficiently and quickly proliferated *in vivo* and the onset bacterial infection in an injured part can be avoided in the course of a treatment. More specifically, a closed cell culture system (1) characterized in that a defection (2) of a tissue on the body surface or inside the body is

tightly sealed to form a closed environment free from the invasion of bacteria, etc. and then a solution appropriate for cell culture is circulated in the tissue defection thus sealed to thereby regenerate the defective tissue; and a method of administering a drug which comprises dissolving a remedy in the perfusion with the use of the above system and thus promoting the treatment of the defection.

FIG.1



EP 1 488 816 A1

Description

CROSS-REFERENCES

[0001] The present invention relates to a system which causes cell tissue of an injured part to proliferate in vivo and induces tissue regeneration of the injured part.

BACKGROUND

[0002] Injured body parts have conventionally been treated by first removing foreign material and other unnecessary material from the injured part, then disinfecting with a disinfectant or the like and covering the injured part with a wound dressing or the like appropriate for treatment in order to achieve self-regeneration of the skin through cell proliferation. However, self-regeneration of skin is plagued with such intractable problems as time-consuming healing, the occurrence of infections in the injured part and the like.

[0003] In order to solve these problems, the method of tissue autotransplantation has recently been applied as a form of regenerative medical engineering, whereby cells near the injured part are collected and proliferated in vitro, and the desired tissue or organ is reconstructed outside the body and returned to the defection. Examples relate to a cell culture system comprising a container for cell proliferation medium and a wound dressing material system (for example, Japanese Patent KOHYO Publication 2001-507218), a cultured skin substitute comprising human fibroblast cells and a product effective for wound treatment (for example, Japanese Patent KOKAI Publication No. 2002-200161) and the like. These methods are all effective when applied to flat tissues, but various problems remain to be solved, including the difficulty of culturing multiple layers of cells (for example, multiple layers of cells including epidermal cells and dermal cells) and the slow speed of cell proliferation.

[0004] In the case of thick tissues, moreover, the cell take rate is not particularly high due to the different tissue structures of cells in the body and the difficulty of supplying oxygen or nutrients because of the lack of blood vessels. In addition, there are difficulties in the case of tissues and injured parts with complex three-dimensional solid structures, and it is impossible to prevent infections from occurring during treatment, so problems which existed in the past still remain to be solved.

[0005] With the foregoing in view it is therefore an object of the present invention to provide a tissue cell culture system for regenerative medical engineering whereby a cell tissue can be efficiently and quickly proliferated on an injured part or in other words in vivo, and whereby the occurrence of bacterial infections in the injured part can be avoided during treatment.

[0006] In order to solve the aforementioned problems, the present inventor perfected the present invention as

a result of exhaustive research when he discovered that it was possible to efficiently and quickly regenerate the tissue of a defection in situ by first removing necrotic tissue and other unnecessary material from the opening of the defection, embedding a bioabsorbable material in the defection space as a foundation for cell proliferation, closing the defection from the outside and circulating a solution appropriate for cell culture in the closed space.

SUMMARY

[0007] Consequently, the invention according to Claim 1, which is the basic mode of the present invention, is a closed cell culture system characterized in that a defection of a tissue on the body surface or inside the body is tightly sealed from the outside to form a closed environment free from the invasion of bacteria, and then a solution appropriate for cell culture is circulated in the tissue defection thus sealed to thereby regenerate the defective tissue.

[0008] That is, the characteristics of the present invention are that the defection itself is sealed from the outside to form a closed area for perfusion of the solution and prevent infection by bacteria, and that a solution appropriate for cell culture is then circulated in that area, and that continuous disinfection and cell culture can be accomplished by altering the components of the perfusion as appropriate.

[0009] Also, because cells ordinarily divide and proliferate by adhering to a particular surface, a foundation to which the cells can adhere is needed for cell proliferation. Cell proliferation is promoted in the present invention by embedding a bioabsorbable material in the defection as such a foundation. Consequently, the present invention according to Claim 2 is a closed cell culture system which promotes regeneration of defective tissue cells by the embedding of a bioabsorbable material in a closed environment in the invention according to Claim 1.

[0010] Examples of such bioabsorbable materials which provided a foundation for cell proliferation include polyglycolic acid fiber, lactic acid glycolic acid copolymer fiber or sponge, glycolic acid caprolactone copolymer fiber, polylactic acid fiber or sponge, lactic acid caprolactone copolymer, polycaprolactone fiber, polydioxane fiber, collagen fiber or sponge, gelatin sponge, fibrin fiber sponge, polysaccharide fiber or sponge, tricalcium phosphate porous beads, calcium carbonate porous beads, hydroxyapatite and the like.

[0011] In the closed cell culture system of the present invention, a solution appropriate for cell proliferation is perfused in a cell culture in a closed environment so that cell proliferation can be accomplished effectively. Consequently, the present invention according to Claim 3 is a closed cell culture system whereby the solution appropriate for cell culture in Claim 1 or 2 is serum isolated from the patient's blood, platelet concentrated serum, a blood preparation, a plasma fraction preparation, a

blood protein fraction component solution, a plasma expander, an osmotic pressure isotonic infusion or a cell culture medium.

[0012] Moreover, the present invention according to Claim 4 is a closed cell culture system whereby a cell proliferation environment appropriate to analgesia and disinfection of the deflection and to each tissue to be regenerated is constructed and programmed by changing the components of the circulating solution at each stage of treatment. That is, in order for cell culture in the deflection to be effective, the most effective closed environment for cell proliferation needs to be established. Consequently, a characteristic of the present invention according to Claim 4 is that a cell proliferation environment suited to analgesia and disinfection of the deflection and to each tissue to be regenerated can be easily constructed in response to these demands.

[0013] Moreover, pH, carbon dioxide partial pressure and oxygen partial pressure of the cell culture environment each vary as the cells are cultured. Consequently, even if a solution of a composition appropriate for cell culture is circulated, the dosed environment still needs to be constantly maintained as an optimal environment for cell culture. To this end, it is desirable that the physical factors of pH, carbon dioxide partial pressure and oxygen partial pressure within the culture liquid of the closed environment be monitored by means of a sensor, the signal whereof is used to control a gas exchanger installed at the inlet of the culture environment, and that gas partial pressure of the closed environment be adjusted in real time according to changes in the culture liquid so as to optimize the cell culture environment. To this end, the present invention according to Claim 5 is a closed cell culture system which incorporates at the inlet to the closed environment a gas exchanger equipped with a monitoring device which measures the pH, carbon dioxide partial pressure and oxygen partial pressure of the closed environment, and optimizes the cell culture environment by automatically adjusting gas concentrations based on signals from the monitoring device.

[0014] The present invention according to Claim 6 is a closed cell culture system wherein the pressure within the closed environment is continuously or intermittently controlled. That is, the pressure in the closed environment can be varied by means of a pressure maintenance device for example, thus ensuring that the space is suitable for growth of nerve tissue or the like in a positive pressure state, or promoting exudation of bodily fluids in a negative pressure state and thus promoting cell proliferation on the surface of an injured part. By applying negative pressure intermittently, moreover, it is possible to uniformly and efficiently replace the circulating liquid in the closed environment by means of a temporary shrinkage in the volume of the closed environment.

[0015] In the case of an extensive and severe burn, a severe loss of body fluid moisture may occur at the injured part. Consequently, when the closed cell culture

system of the present invention is used for cell culture of an injured part, the solution which is circulated as the perfusion must have its osmotic pressure adjusted so that the injured surface of the burn can be appropriately maintained. To this end, the present invention according to Claim 7 is a closed cell culture system equipped with a circuit for circulating a perfusion the osmotic pressure of which has been adjusted externally.

[0016] Moreover, when the closed cell culture system of the present invention is used for cell culture of an injured part, and the injured part involves a relatively deep injury such as nerve cell rupture or the like, less invasive access is desirable when culturing such nerve cells or vascular cells. To achieve such access, a puncture needle can be adopted. Consequently, the present invention according to Claim 8 is a closed cell culture system of an aspect wherein a cell culture space is created within an organ or deep inside the body cavity by a combination of a puncture device and a balloon catheter.

[0017] That is, proliferation of nerve tissue cells or vascular tissue cells requires that a space be maintained in which they can proliferate. To create such a space the target site in the body is reached by a combination of a puncture needle and a balloon catheter which is passed through the lumen, and the balloon is inflated to create a space suited for proliferation of the target cells. This is also characterized in that it can effectively deposit a bioabsorbable material at the injured part.

[0018] It is also possible in the case of the liver, pancreas, kidneys and other parenchymal organs to create a culture space by the same methods and culture the target cells within each organ, thus proliferating within an organ with dysfunction or depressed function cells having better functional structure than cells cultured by external cell culture techniques.

[0019] The present invention also provides a method capable of administering a drug which is effective for purposes of cell culture in an injured part. That is, in the closed cell culture system provided by the present invention, the optimal culture components for proliferation of the various cells can be substituted, and cell proliferation factors and vascular proliferation factors which further promote healing can be added to the perfusion and perfused in the deflection. Consequently, the present invention according to Claim 9 is also a method of administering a drug by dissolving a therapeutic drug in a perfusion using the closed cell culture system according to Claim 1 and thus promoting treatment of a deflection. In order to prevent problems due to mixture of incompatible drugs when the therapeutic drug is changed, an intermittent negative pressure cycle is applied to the closed system in such cases so that the perfusion circulating through the cell culture space in the closed environment is actively replaced, allowing the drug solution to be uniformly and efficiently exchanged.

[0020] Drugs which can be administered in this case include disinfectants, local anesthetics, anti-phlogistics and analgesics, antibiotic preparations, peripheral va-

sodilators, various cell proliferation factor preparations, nerve proliferation factor preparations, vascular proliferation factor preparations (or various cell proliferation suppression factor preparations when cancer cells are removed), immunosuppression preparations and the like.

[0021] In addition to such drugs, various cell adhesive molecules such as fibronectin, hydnoractin and the like can be added to the perfusion. Genes can also be introduced, and examples of such genes include naked DNA, adenovirus vector genes, retrovirus vector genes, liposome encapsulated genes, hydrogel encapsulated genes and the like.

[0022] The invention according to Claim 10, which is a different mode of the present invention, is a method of cell transport and proliferation whereby cells from the patient are first collected, cultured and proliferated in vitro, then suspended in a perfusion and returned to the closed culture system via the perfusion system so as to provide therapy by means of a cell culture of the injured part while promoting healing of the injured part in conjunction with culture proliferation and take by tissue cultured in vitro.

DESCRIPTION OF DRAWINGS

[0023]

Figure 1 is a typical simplified exploded view for explaining the closed cell cultured system of the present invention.

Figure 2 is a simplified cross-section showing the closed cell culture system of the present invention accessed from the body surface.

Figure 3 explains the concept of cell proliferation by the system of the present invention for cell proliferation in the case of nerve cell rupture, which is a deep internal injury.

Figure 4 explains the concept of cell proliferation by the system of the present invention for cell proliferation in the case of nerve cell rupture, which is a deep internal injury.

Figure 5 explains the concept of cell proliferation by the system of the present invention for cell proliferation in the case of nerve cell rupture, which is a deep internal injury.

Figure 6 explains the concept of cell proliferation by the system of the present invention for cell proliferation in the case of nerve cell rupture, which is a deep internal injury.

Figure 7 is a typical explanatory drawing of a case of multiple perfusions circulated by means of the closed cell culture system of the present invention.

Figure 8 is a typical explanatory drawing of a case in which a perfusion is circulated by the closed cell culture system of the present invention using a constant pressure continuous suction device, an infusion bag and an infusion tube circuit.

Key

[0024]

- | | | |
|----|----|---|
| 5 | 1 | Closed cell culture system |
| | 2 | Defection in body tissue |
| | 3 | Surgical film |
| | 7 | Bioabsorbable material to be embedded |
| | 20 | Closed cell culture system |
| 10 | 23 | Dressing film |
| | 24 | Double discs |
| | 25 | Foundation biomaterial |
| | 26 | Cell proliferation site |
| | 30 | Puncture needle |
| 15 | 32 | Balloon |
| | 33 | Bioabsorbable material |
| | 34 | Double lumen catheter |
| | 40 | Perfusion bag |
| | 41 | 3-way stopcock |
| 20 | 42 | Flow rate regulator |
| | 43 | Closed cell culture container |
| | 44 | Pump |
| | 45 | Discharge |
| | 46 | Patient |
| 25 | 47 | pH and gas monitor |
| | 48 | Gas exchanger |
| | 51 | Constant pressure continuous suction device |

DETAILED DESCRIPTION

[0025] The closed cell culture system provided by the present invention is explained in detail below with reference to the drawings.

[0026] Figure 1 is a typical simplified exploded view explaining the closed cell cultured system of the present invention. That is, fundamentally the closed cell culture system (1) provided by the present invention is characterized in that a defection (2) of a tissue on the body surface or inside the body is tightly sealed to form a closed environment free from the invasion of bacteria, etc. and then a solution appropriate for cell culture is circulated in the tissue defection thus sealed to thereby regenerate the defective tissue.

[0027] More specifically, in the closed cell culture system (1) provided by the present invention (which in the figure is shown not closed, but in a typical expanded view) a closed environment free from the invasion of bacteria and the like is created by means (3) (a surgical film or other adhesive sheet in the figure) which seals defection (2) of a tissue on the body surface or inside the body, namely by covering and sealing defection (2) of a tissue on the body surface with surgical film (3) to create a closed environment, and a solution appropriate for cell culture is introduced into the closed tissue defection (2) through perfusion introduction tube (10) (in the direction of arrow A in the figure) and perfused to perfusion discharge tube (11) (in the direction of arrow B in the figure).

[0028] The perfusion which is perfused through system (1) by perfusion introduction tube (10) soaks for example into epidermal bioabsorbable material (6) via disc 2 (5), from whence it further soaks into embedded bioabsorbable material (7), which is embedded in the tissue defection (2) below, providing the cells in tissue defection (2) with an environment suited for cell proliferation so that cell proliferation can proceed efficiently.

[0029] Subsequently, the perfusion which has soaked into embedded bioabsorbable material (7) to fill tissue defection (2) passes through the space between disc 1 (4) and disc 2 (5) and is discharged through perfusion discharge tube (11) on disc 1 (4), completing perfusion.

[0030] The two disc layers, disc 1 (4) and disc 2 (5) in the system, may be any with a structure capable of holding perfusion introduction tube (10) and perfusion discharge tube (11), respectively, and they may be formed as a whole with the tubes from a material such as non-water-permeable plastic or the like.

[0031] As explained above, by circulating a perfusion into the closed environment of a tissue defection through system (1) of the present invention, it is possible to efficiently promote cell proliferation in the tissue defection by means of a bioabsorbable material which provides a foundation for cell adhesion, and once cell proliferation is complete the bioabsorbable material which forms the foundation is broken down and absorbed and the tissue of the defection is regenerated under sterile conditions.

[0032] Figure 2 is a simplified cross-section showing the closed cell culture system (20) of the present invention accessed from the body surface. That is, in the case of an external injury, burn, bed sores or other injury, bodily tissue including the epidermis (21) and dermis (22) is defective, and the injured part is exposed at the body surface. In this case, the 2-disc wound surface adapter (24) of the closed cell culture system (20) of the present invention together with a dressing film (23) is applied to the surface of the injury, and a perfusion is perfused. Even in this case, a cell proliferation site (26) having an embedded bioabsorbable material as a suitable foundation biomaterial (25) can be created to promote tissue proliferation in tissues requiring tissue proliferation.

[0033] In this case, the perfusion is perfused into the injured part through perfusion inlet (28) in the center of the closed tissue culture system (20) to reach the foundation biomaterial (25) which is a bioabsorbable material, around which it is dispersed. It then passes from the periphery (27) through the gap between the two discs (24), and is expelled as discharge from perfusion outlet (29).

[0034] Consequently, invasion of bacteria or the like is prevented because the surface of the injury is sealed, and cell proliferation in the injured part is effectively achieved through the use of a perfusion and a foundation biomaterial.

[0035] The size of the closed tissue culture system of the present invention for accessing the surface of the

body can be designed at will to match the size of the individual injured part. Consequently, in the case of an extensive burn or the like, a combination of multiple closed tissue culture systems of the present invention having two disc layers can be matched to the size and shape of the burn, and perfusion perfused through each system simultaneously to improve effectiveness as in the case of single use.

[0036] In cases such as nerve cell rupture or rupture of vascular tissue in which cell proliferation is performed in an injured part relatively deep in the body, a closed cell culture system employing a puncture needle is employed because less invasive access is desirable. Specifically, a space for proliferation of nerve tissue cells or vascular tissue cells can be created through a combination of a puncture needle and a balloon catheter which is passed through the lumen, after which the foundation biomaterial is deposited in the site expanded by the balloon and perfusion is circulated through a perfusion catheter. The puncture needle in this case is constructed so as to be capable of bending flexibly and of depositing the catheter according to the injured part where cells are to be proliferated.

[0037] The concept of cell proliferation in the system of the present invention for proliferating cells in an injured part relatively deep in the body, such as a nerve cell rupture, vascular tissue rupture or the like, is shown in Figures 3 through 6. That is, a puncture needle (30) such as that shown in Figure 3 is inserted through the skin surface, the tip is guided to the part where a space for cell proliferation is to be constructed, a balloon catheter (31) is exposed through the tip of the puncture needle as the tip of the puncture needle is withdrawn, and balloon (32) is inflated.

[0038] Next, once a space (space in tissue) for cell proliferation has been created by inflation of the balloon, the balloon is deflated and only the balloon catheter is withdrawn as shown in Figure 4. A bioabsorbable material (33) which will provide a foundation for cell proliferation is then inserted through the lumen of puncture needle (30). The bioabsorbable material can also be placed via the puncture needle using a catheter already present in the lumen.

[0039] Once the bioabsorbable material which provides a foundation for cell proliferation has been deposited in the injured part and the catheter for embedding the bioabsorbable material has been withdrawn, a double lumen perfusion catheter (34) for supplying perfusion appropriate to cell proliferation is inserted as shown in Figure 5, the puncture needle is withdrawn from the body leaving only the perfusion catheter (34), the perfusion inlet (35) and outlet (36) are attached to the perfusion system, and perfusion is initiated. A typical view of this situation is shown in Figure 6.

[0040] As described above, by using the closed cell culture system of the present invention it is possible to tightly seal a defection of a tissue on the body surface or inside the body to form a closed environment free

from the invasion of bacteria, and proliferate cells in the defection by circulating a solution appropriate for cell culture in the tissue defection thus sealed, thereby regenerating the tissue.

[0041] Moreover, in the closed cell culture system provided by the present invention it is possible by varying the components of the circulated solution at each stage to construct a cell proliferation environment suited to analgesia and disinfection of the defection and to each tissue to be regenerated. For example, a method which can be used therefor, as shown in the typical simple view of Figure 7, is to pass multiple types of cell culture perfusion from multiple containers (40) (2 in the figure) through a 3-way stopcock (41) capable of switching the type of perfusion, controlling the flow rate by means of a flow rate regulator (42), and once it has perfused through a closed cell culture system (closed culture container) (43) on the patient (46), discharge the perfused culture liquid via a pump (44) to a discharge container (45).

[0042] In this case, the discharge rate of the pump can be controlled using a pressure sensor for example, thus maintaining a constant pressure in the target injured part where cell proliferation is performed.

[0043] When multiple perfusions are used, it is possible not only to simply perfuse multiple components appropriate to cell proliferation, but also to perfuse disinfectants for disinfecting a contaminated wound or antibiotics for suppressing proliferation of bacteria at the initial stage, and also to supply perfusion containing various cell proliferation factors, nerve proliferation factors, vascular proliferation factors (or various cell proliferation suppressing factors when cancer cells have been extracted) and the like.

[0044] A perfusion containing local anesthetics, antipruritics and analgesics, antibiotics, peripheral vasodilators, immune suppressors and the like can also be perfused to achieve effective therapy.

[0045] Moreover, the pH, carbon dioxide gas partial pressure and oxygen partial pressure within the culture environment all vary as cell culture progresses. Consequently, the closed environment which is the culture environment should constantly be maintained as an optimal environment for cell culture. To this end, a monitor (47) is provided which measures and monitors physical factors in the culture liquid in the closed environment, namely pH, carbon dioxide gas partial pressure and oxygen partial pressure by means of a sensor, and a gas exchanger (48) installed at the inlet to the culture environment is controlled by means of signals from the monitor, so that the gas partial pressure of the closed environment can be adjusted in real time according to changes in the culture liquid, thus optimizing the cell culture environment.

[0046] The closed cell culture system provided by the present invention is also capable of perfusing a perfusion appropriate for cell culture using a constant pressure continuous suction device, infusion bag and infu-

sion tube circuit commonly used in hospitals. A typical simple view of this is shown in Figure 8. The symbols in Figure 8 are used in the same way as in Figure 7.

[0047] In Figure 8, the basic concept is the same as that of the system shown in Figure 7, with the difference that the part which discharges perfused culture liquid to a discharge container via a pump in Figure 7 is replaced by a part connected to a constant pressure continuous suction device (51) commonly used in hospitals.

[0048] As described above, with the present invention once necrotic tissue and other unnecessary matter has been removed from the opening of a defection, a bioabsorbable material which provides a foundation for cell proliferation can be embedded in the defective space and the defection closed off from the outside, and by circulating a solution appropriate for cell culture through the closed space it is possible to efficiently proliferate cells of the defection in situ and regenerate tissue.

Examples

[0049] Specific cases of cell proliferation of the present invention are explained below through examples.

Example 1: Treatment of an injured part after graft skin harvesting surgery

[0050] Bleeding from the wound surface after harvesting was quickly stopped using fibrin spray, and the periphery was disinfected. Next, after the concave area was filled with collagen beads an open cell collagen sheet having holes in the middle was formed somewhat larger than the wound surface and placed over the wound surface. A two-layer adapter was also placed on top after being shaped in the same way as the collagen sheet, and a surgical film having openings only at the inlet and outlet of the adapter was applied to form a closed space of the collagen beads and sheet. When there was pain at the harvesting site, the local anesthetic xylocaine was circulated through the closed space together with a plasma preparation containing a mixture of three antibiotics, and three hours later the local anesthetic and antibiotics were stopped and perfusion was switched to basic fibroblast growth factor (bFGF).

[0051] Dermal layer tissue was harvested separately, and fibroblast cells which had been carefully float cultured in vitro in fetal cow serum were perfused in exchange for patient serum. Cell transplantation was performed by injecting this opaque cell solution into the closed culture container through the lower 3-way stopcock of a perfusion bag. Next, once cell proliferation of the dermal cells was complete epidermal growth factor (EGF) was substituted for the bFGF, and epidermal cells were cultured until they matched the surrounding skin surface.

Example 2: Neural reconstruction of a ruptured nerve of the thigh

[0052] A puncture needle capable of bending flexibly was inserted so as to reach both ruptured ends of a ruptured nerve. A balloon was selected of a length to reach both ruptured ends of the nerve, a balloon catheter was inserted through the puncture needle and the balloon inflated to create a space between the ruptured ends of the nerve.

[0053] A bundle of parallel collagen fibers to provide a foundation for nerve growth in the space was inserted by a catheter contained in a lumen. A fine perfusion catheter was then substituted for the catheter and the puncture needle was withdrawn, leaving only the perfusion catheter.

[0054] 10-8 moles of nerve growth factor were added to serum obtained from the patient's blood, perfusion was initiated, and a circulating circuit was substituted at the point at which no more air came out.

[0055] A slight positive pressure was maintained during perfusion so that the space between the severed ends of the nerve would not collapse. Once the cells had grown sufficiently the perfusion catheter was withdrawn to complete the process.

Example 3: Treatment of a severe burn

[0056] After skin, dead tissue and the like had been excised from a severe burn on the abdomen, the surface of the burn was covered with a collagen fiber sheet, and a 2-layer perfusion adapter was then attached thereupon. The surface of the wound was first disinfected with an isotonic plasma extender containing povidone-iodine.

[0057] A human plasma preparation with osmotic pressure made similar to that of the burn surface was perfused to suppress loss of bodily fluid by the patient from the burn surface. An antibiotic injection was also added to prevent infection, and if the patient complained of pain the local anesthetic xylocaine was injected into the perfusion for pain relief.

[0058] Once the patient's condition had stabilized administration of xylocaine and antibiotics was stopped and fibroblast growth factor (bFGF), platelet derived growth factor (PDGF), epidermal growth factor and other cell growth factors were added to the perfusion. Once the epidermis had sufficiently proliferated on the wound the closed cell culture container was removed and the wound surface exposed to air while awaiting further healing.

Example 4:

[0059] A catheter introducer equipped with a puncture needle was inserted into a rabbit liver under observation by ultrasound tomography. The puncture needle was withdrawn, leaving the introducer sheath, a Fogarty

thrombus removal balloon catheter was inserted in its place, and the balloon was inflated to a diameter of about 2 cm to secure a space for cell culture in the liver. The balloon was deflated, the Fogarty catheter was withdrawn, and a teflon tube containing cottony collagen fiber pellets was inserted into the culture space, which was then filled with the collagen fiber pellets. The teflon tube for inserting the collagen was withdrawn, a double lumen for perfusion was placed so that the perfusion openings were in the culture space, and the catheter introducer sheath was withdrawn leaving only the double lumen catheter, which was fixed to the skin by suturing and also by external catheter fixing film.

[0060] Serum prepared from the blood of the same rabbit was perfused into the cell culture space while liver cells were cultured inside the rabbit liver for 30 days.

[0061] After 30 days of treatment, the rabbit was sacrificed, and autopsy of the treated liver revealed proliferation of liver cells within the collagen fibers.

[0062] As described above, an advantage of the closed cell culture system of the present invention is that a defection is closed off from the outside to prevent infection by bacteria or the like, and a solution appropriate for cell culture is circulated in the closed part to efficiently proliferate cells of the defection in situ so that tissue can be regenerated.

[0063] Another advantage is that by varying the perfusion, growth factors or therapeutic drugs can be administered to the target site, thus ensuring more effective tissue regeneration.

Claims

1. A closed cell culture system **characterized in that** a defection of a tissue on the body surface or inside the body is tightly sealed from the outside to form a closed environment free from the invasion of bacteria, and a solution appropriate for cell culture is circulated in the tissue defection thus sealed to thereby regenerate the defective tissue.
2. The closed cell culture system according to Claim 1, wherein a bioabsorbable material is embedded in the closed environment to promote regeneration of the defective tissue cells.
3. The closed cell culture system according to Claim 1 or 2, wherein the solution appropriate for cell culture is serum isolated from the patient's blood, a blood preparation, platelet concentrated serum, a serum fraction preparation, a blood protein fraction component solution, a plasma expander, an osmotic pressure isotonic infusion or a cell culture medium.
4. The closed cell culture system according to any of Claims 1 through 3, wherein a cell proliferation en-

vironment suited to analgesia and disinfection of the deflection and to each tissue to be regenerated is constructed and programmed by varying the components of the circulating solution at each stage of treatment.

5

5. The closed cell culture system according to any of Claims 1 through 4, wherein a gas exchanger connected to a monitoring device which measures pH, carbon dioxide partial pressure and oxygen partial pressure in the closed environment is incorporated into a circuit connected to the closed environment, and the cell culture environment is optimized by automatically adjusting the gas concentrations based on signals from the monitoring unit. 10 15
6. The closed cell culture system according to Claim 1, wherein the pressure inside the closed environment is continuously or intermittently controlled. 20
7. The closed cell culture system according to Claim 1, comprising a circuit for perfusing a perfusion the osmotic pressure of which has been adjusted externally. 25
8. The closed cell culture system according to Claim 1 or 2, wherein a cell culture space is created within an organ or deep inside the body cavity through a combination of a puncture device and a balloon catheter. 30
9. A method of administering a drug which promotes treatment of a deflection wherein a therapeutic drug is dissolved in a perfusion using the closed cell culture system according to Claim 1. 35
10. A method of cell transport and proliferation for promoting healing of the injured part, whereby cells from the patient are first collected, cultured and proliferated in vitro, then suspended in a perfusion and returned to the closed culture system via the perfusion system so as to provide therapy by means of a cell culture of the injured part while, in conjunction therewith, culture proliferation and take are performed by adding tissue cultured in vitro. 40 45

50

55

FIG.1

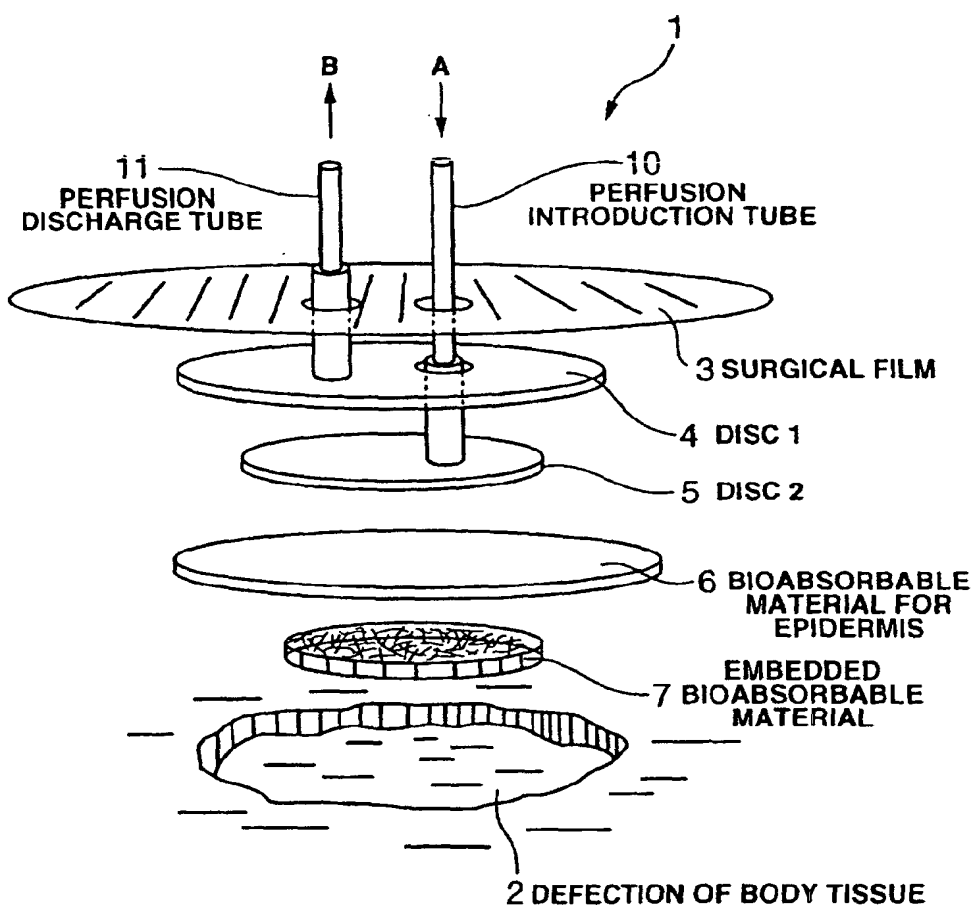


FIG.2

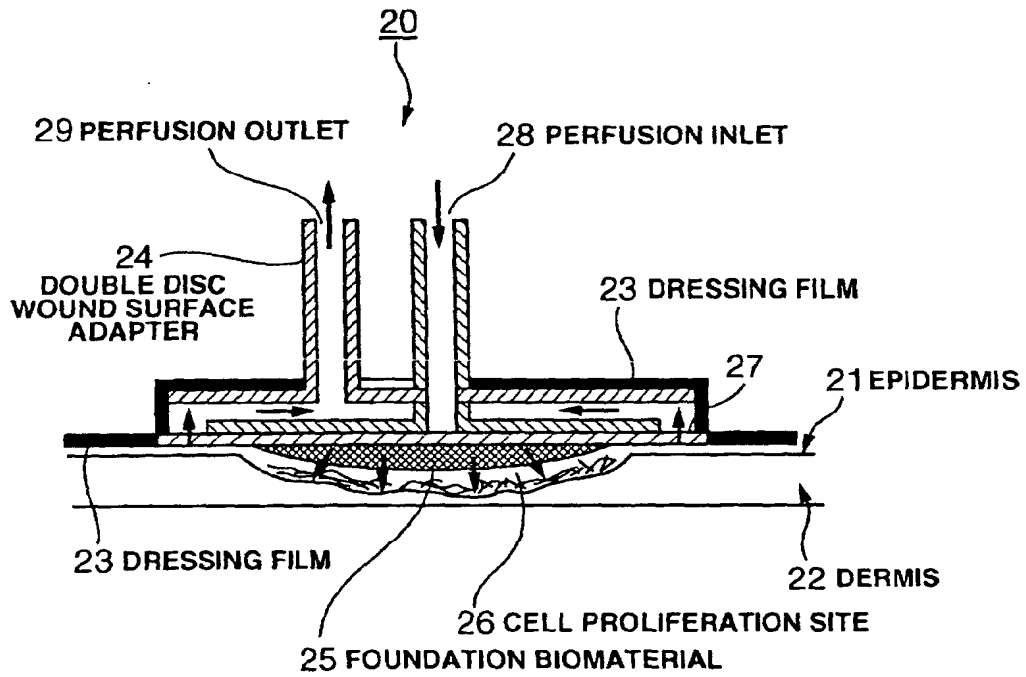


FIG.3

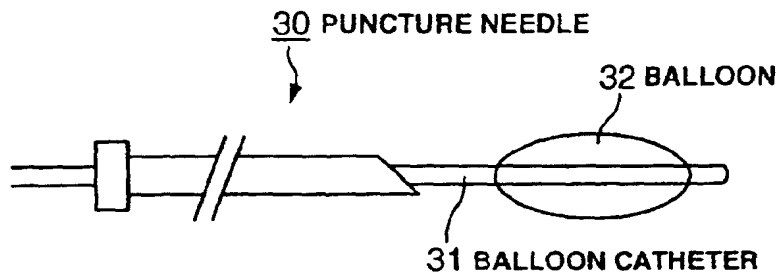


FIG.4

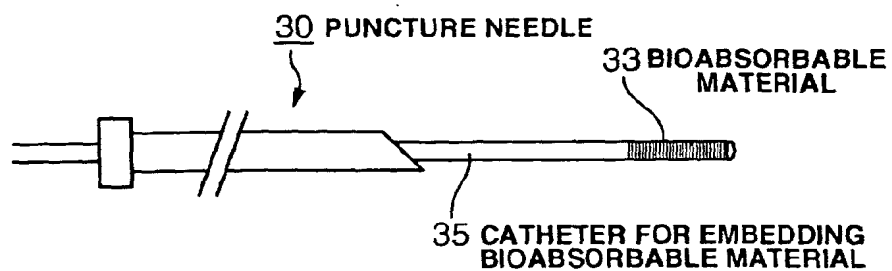


FIG.5

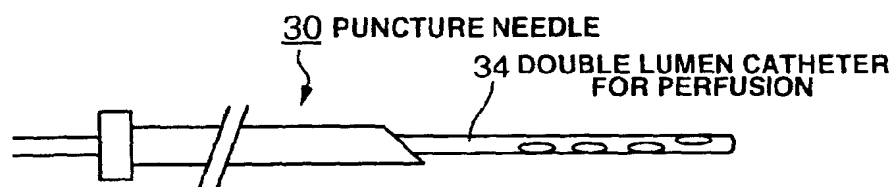


FIG.6

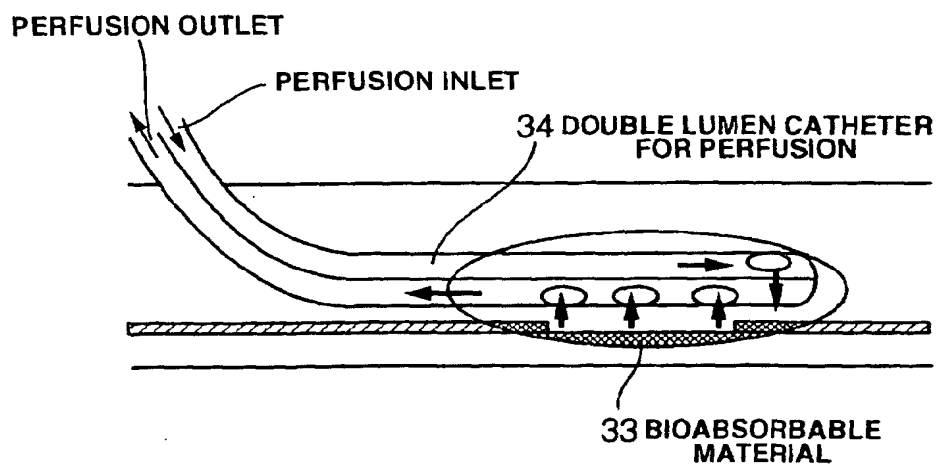


FIG.7

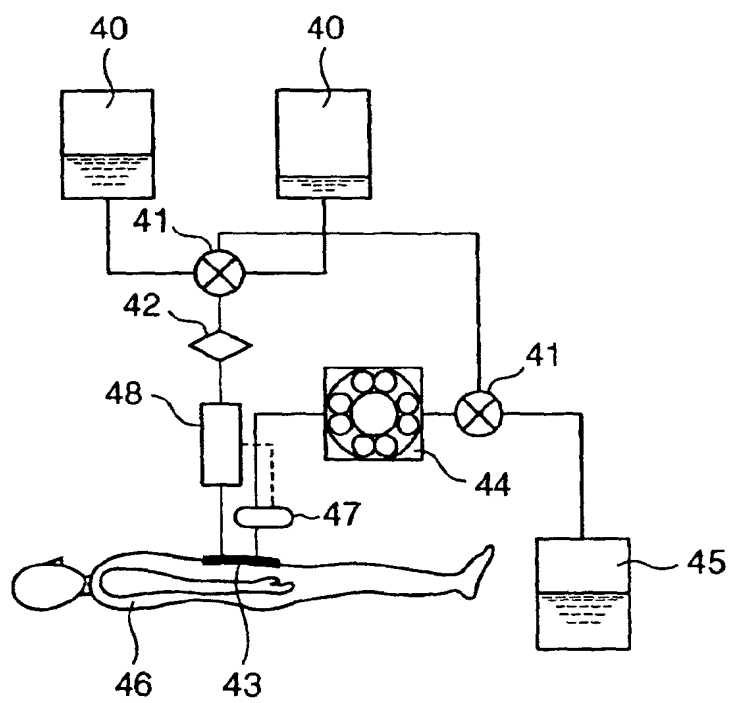
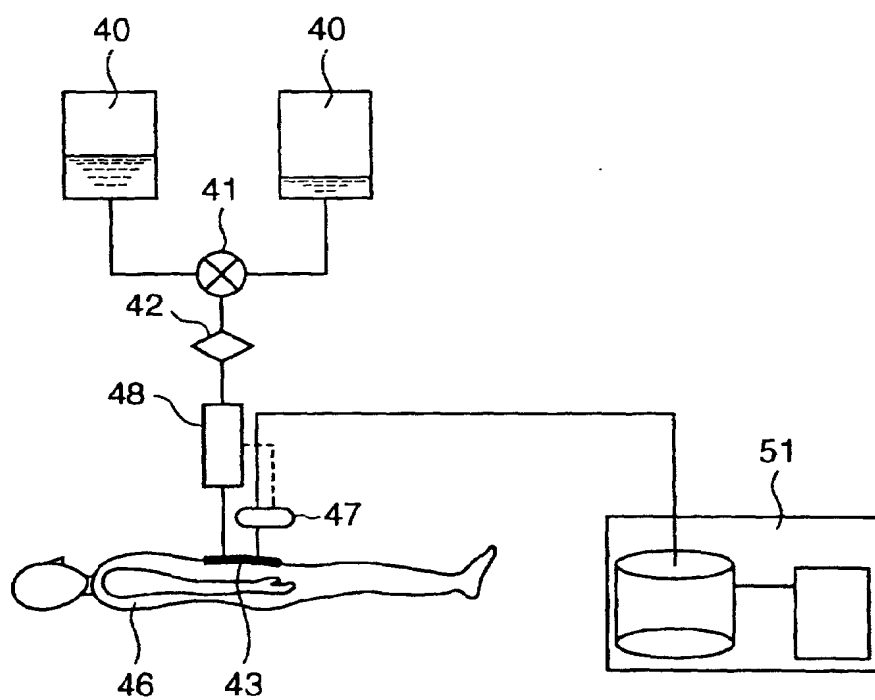


FIG.8



INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/02501

A. CLASSIFICATION OF SUBJECT MATTER

Int.Cl.⁷ A61L27/38 // C12N5/00, C12M3/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

Int.Cl.⁷ A61L27/38 // C12N5/00, C12M3/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Jitsuyo Shinan Koho	1926-1996	Jitsuyo Shinan Toroku Koho	1996-2002
Kokai Jitsuyo Shinan Koho	1971-2002	Toroku Jitsuyo Shinan Koho	1994-2002

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA/MEDLINE/BIOSIS/EMBASE (STN), WPI/L (QUESTEL), JICST FILE (JOIS)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	WO 95/31157 A1 (THM BIOMEDICAL, INC.), 23 November, 1995 (23.11.95), Claims 73, 79; page 7, lines 19 to 23; page 28, lines 18 to 28 & AU 9525905 A & EP 759731 A1 & JP 10-500589 A & US 5855608 A & US 5981825 A	1-4 5-7
X Y A	WO 99/07276 A2 (THERAPEUTIC MICRODIALYSIS CORP.), 18 February, 1999 (18.02.99), Claims 21, 28; page 55, lines 24 to 29 & AU 9887738 A & US 6030358 A & EP 1009276 A2 & JP 2001-513349 A	1-4 5-7 8

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
28 April, 2003 (28.04.03)Date of mailing of the international search report
20 May, 2003 (20.05.03)Name and mailing address of the ISA/
Japanese Patent Office

Authorized officer

Facsimile No.

Telephone No.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/02501

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 2001/0021529 A1 (Takagi Industrial Co., Ltd.), 13 September, 2001 (13.09.01), Claims 9, 26 & WO 01/64848 A1 & JP 2001-238663 A & AU 200136002 A & US 2002/0037586 A1 & US 2002/0098586 A1	5, 6
Y	JP 11-046759 A (Terumo Corp.), 23 February, 1999 (23.02.99), Par. Nos. [0018], [0023] (Family: none)	7
A	WO 01/87271 A1 (DAVID, James, Johnson), 22 November, 2001 (22.11.01), Full text & AU 200153411 A & US 6471685 B1	1-7

Form PCT/ISA/210 (continuation of second sheet) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/02501

Box I Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

- 1.
- ☒
- Claims Nos.: 9, 10

because they relate to subject matter not required to be searched by this Authority, namely:

Claims 9 and 10 pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

- 2.
- ☐
- Claims Nos.:

because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

- 3.
- ☐
- Claims Nos.:

because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest ☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (1)) (July 1998)

INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP03/02501

Claims 1 to 8

The inventions as set forth in the above claims, a "closed cell culture system" is specified merely by procedures, i.e., "tightly sealing a defection of a tissue on the body surface or inside the body", "circulating a solution appropriate for cell culture" or "regenerating the defective tissue". Therefore, it is unknown whether the claimed inventions are inventions of product or inventions of process.

Provided that these inventions are inventions of process, the inventions according to the above claims of the present case pertain to methods for treatment of the human body by therapy and thus relate to a subject matter which this International Searching Authority is not required, under the provisions of Article 17(2)(a)(i) of the PCT and Rule 39.1(iv) of the Regulations under the PCT, to search.

In this report, therefore, an examination was made by referring these inventions as to inventions of product. In this case, the specific structures of the products involved in the above claims cannot be specified. Thus, these claims fail to fulfill the requirement of clearness as defined in PCT Article 6.